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Calcium Phosphate Cement Containing Strontium for Increasing Bone Formation: In Vitro and In Vivo Study

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Abstract— **The purpose of this research was to examine the performance of a 3 wt% nano-strontium hydroxyapatite (10% of calcium in hydroxyapatite was replaced with strontium) additive with calcium phosphate cement (CPC) in vitro and in vivo for increasing bone formation. The phase composition, thermal analysis, microstructure, setting time of the prepared calcium phosphate cement was evaluated. Also, the in vitro testing of MTT assay and alkaline phosphatase (ALP) activities, and in vivo testing of radiological and histological examinations between three groups of 3 wt% Sr-HA/CPC, CPC and control were characterized and compared. X-ray diffraction (XRD) ascertained that increasing the ratio of Powder/Liquid (P/L), crystallinity of the prepared cement has increased. The substitution of strontium instead of calcium in CPC can also alter the crystal structure, including some structural disorder. However, in the CPC with no strontium hydroxyapatite (Sr-HA), no significant increase in crystallinity was observed. SEM observations showed CPC with increasing P/L ratio, the formation of hydroxyapatite crystals arising from the interaction of solid and liquid phase of cement was decreased. Also, incorporation of Sr within Ca site culminates in a dramatic increase in crystallinity of hydroxyapatite. In vitro biological properties demonstrated that addition of 3 wt. % Sr-HA into CPC increased MTT assay and ALP activity increased, which may be due to the presence of strontium ions. Finally, histological study cleared that greater remodeling were observed at 4 weeks after implantation when the 3 wt% Sr-HA/CPC was employed. Finally, the obtained results ascertained that CPC can be a potential candidate as a carrier with strontium additives for bone regeneration.**

Index Terms— **Calcium phosphate cement (CPC); Strontium; Hydroxyapatite; Brushite; Bone formation**

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I. INTRODUCTION

In 1980s Calcium phosphate cements (CPC) were discovered by LeGeros and Chow et al [1]. Calcium phosphate cements are used as the bone substitute materials and can serve as injectable pastes to fill defects and are biocompatible and osteoconductive [2, 3]. A bioactive material is one that can bind with the surrounding bone without the formation of fibrous tissue [4]. Bioactivity, together with the perfect adaptability of the cement paste, leads to a stable connection between defect and implant and boost bone healing process [5, 6]. The main reaction of calcium phosphate cements is the cementing action of acidic and basic calcium phosphate compounds in an aqueous solution [1, 2, 7]. In another word calcium phosphates are formed by a chemical reaction between two phases. The dry powder phase is a combination of calcium orthophosphate and the liquid phase is water or a calcium or phosphate-containing aqueous solution [1, 2, 4, 7, 8]. After mixing of the powder phase with the liquid phase a paste forms that sets and hardens into a solid mass. One of the advantages of calcium phosphate cements is that no heat is generated during cementation reaction and the cementation process is not exothermic so there is no risk of hyperthermia while using them in the human body for the surrounding tissue [2, 3, 7, 9, 10]. Furthermore calcium phosphate cements are intrinsically microporous. These porosities are left by extra aqueous solution after hardening and can be used for carrying of biological fluids into CPCs and causes degradation and replacement of CPCs by bone. Degradation rate depends on the composition and microstructure of the cement. Also Degradation products are well absorbed in physiological environment [11-14].

Despite having many advantages, calcium phosphate cements have also some issues which include poor mechanical properties. Like most ceramics, these types of cement are brittle and due to their intrinsic porous structure, their strength is lower than acrylic cements. This weakness has made their applications limited to moderate bearing situations [11, 15-17].

There are two main calcium phosphate cement final product: hydroxyapatite and dicalcium phosphate dehydrate (brushite). The final cement formed by the liquid and solid phase,

depends on pH of the solution. Hydroxyapatite forms for pH higher than 4.2 and for lower pH amounts, brushite (DCPD) is obtained. Solubility of brushite is greater than hydroxyapatite at physiological pH. In fact brushite is metastable under physiological conditions and can be resorbed more quickly than hydroxyapatite [2, 8, 18].

Brushite cements have generally too short setting time ranging between 30 and 60 s that limits their use orthopedic applications. To make these cements suitable for orthopedic usage, specific setting retardants, such as pyrophosphate ions or citrates are usually added to slow down the setting process [7, 16, 19, 20]. Their effects generally consist in inhibiting nucleation and growth of calcium phosphate crystals. Furthermore some ions like Sr, Zn, and Mg can serve as enzyme cofactors in bone regeneration process [21-23]. Thus, incorporation of these ions in biomaterials can have big impact in bone tissue healing as the use of growth factors [24, 25].

Strontium can stimulate osteoblast differentiation and inhibit osteoclast activity and is a valuable ion in the treatment of osteoporosis so there are interests of incorporating strontium in calcium phosphate cements. Incorporation of strontium affects the reactivity of the cement but can also modify the final composition of the material [24-26].

Therefore, in this study, the structural, physicochemical and biological properties of the type of calcium phosphate cements modified by strontium ion were evaluated.

II. Experimental procedure

2.1. Preparation of CPC

To prepare calcium phosphate cement powder in this research, one gram of tetracalcium phosphate (Ca4P2O9, TTCP)- with a controlled mean particle size of 10 µm was synthesized following the method from the reaction of dicalcium phosphate dihydrate (CaHPO4.2H2O) and calcium carbonate (CaCO3)- was mixed with one gram of dicalcium phosphate dehydrate (CaHPO4.2H2O, DCPD; Merck Co.) and then 1.205 ml (P/L=0.83), 0.8 ml (P/L=1.25) and 0.645 ml (P/L=1.55) of disodium hydrogen phosphate (Na2HPO4; Merck Co.) was added to the mixture respectively in order to set the cement. Finally, 3 wt. % of synthesized nano-strontium substituted hydroxyapatite (Sr-HA) (10% of calcium in hydroxyapatite was replaced with strontium) was added respectively to the prepared cement. The calcium phosphate cement powder was mechanically ground to the mean particle size distribution of 3 mm, vacuum-packed and g-ray-sterilized (20 kGy).

2.2. Characterization of CPC

2.2.1. X-ray diffraction analysis

XRD patterns of the prepared cements were obtained at room temperature using an INEL Equinox3000 (Cu-Kα radiation) operating at a voltage of 40 kV and current of 30 mA. CPCs were analyzed in the 2θ angle range of 0–80° and their patterns were studied to determine the crystal phases present in the samples.

2.2.2. Fourier transform infrared spectroscopy analysis

Infrared spectroscopy was carried out to determine the chemical composition of the samples using FTIR NICOLET USA operating in the wavenumber range of 400–4000 cm-1 and used in the absorption mode.

2.2.3. Simultaneously thermal analysis analyses

Simultaneously thermal analysis (STA) generally refers to the simultaneous application of thermogravimetry (TGA) and differential scanning calorimetry (DSC) to one and the same sample in a single instrument. A thermoanalyzer (STA; Polymer Laboratories PL-STA 1640) that was started from room temperature up to ~ 1000°C with the heating rate of 10°C /min was used to record the conventional thermoanalytical curves.

2.2.4. Scanning electron microscopy (SEM)

The cement samples were coated with a thin layer of Gold (Au) by sputtering (EMITECH K450X, England) and then the microstructure of the cement samples were observed in a scanning electron microscope (SEM; AIS-2100 780 SERON SOUTH KOREA) that operated at an acceleration voltage of 20 kV.

2.2.5. Setting time measurement

The setting time is the time when the CPC paste loses its plasticity and starts to harden to form a solid mass. The powders of TTCP / DCPD were mixed with ratios of 1:1 and then were mixed with liquid phase for a minute at room temperature. Finally Cement mixture was poured in a plastic mold with dimensions of 15 × 15 mm2 to set. Setting time of the prepared cement was measured using Vicat test according to ASTM-C-18798 standard.

2.2.6. Biological evaluation

2.2.6.1. In vitro study

2.2.6.1.1. MTT assay

For measuring the cell viability of the prepared samples MTT (3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was used. At first, under standard culturing conditions SAOS-2 cells were seeded on to 96 well plates at a density of 1×104 cells per well and were incubated. The cells were incubated on the samples for 3 and 7 days. After the incubation, the medium was removed and the media containing 10% of MTT solution was added. Then, the plates were incubated at 37 8C for 4 h. The medium was then removed and 100 µl of solubilization buffer (Triton-X 100, 0.1N HCl and isopropanol) were added to each well to dissolve the formazan crystals, which have been produced due to the activity of living cells in MTT solution. The absorbance of the lysate was measured in a microplate reader at a wavelength of 570 nm.

2.2.6.1.1.2. Alkaline phosphatase activity

Alkaline phosphatase (ALP) is an enzyme whose production signifies proliferation and differentiation of osteoblasts. An ALP assay kit was used to measure ALP activity according to the manufacturer's protocol (Biocat, Heidelberg, Germany). Briefly, human osteosarcoma cell lines (SAOS-2) were seeded in 24-well cell culture plates at a density of 1×104cells/cm2. The glass samples (n=5) were placed in the wells, Three wells in the absence of glass samples were used as negative controls. The plates were incubated for 3 and 7 days at 37 ºC in humidified air with 5% CO2 with half media.

Then, the supernatant of each well was removed and the cell layer was rinsed twice with PBS, homogenized with 1 ml Tris buffer, and sonicated for 4 min on ice. Aliquots of 20 ml were incubated with 1ml of a p-nitrophenyl phosphate solution at 30ºC for up to 5 min. Cellular alkalinephosphate activity was determined by the conversion of p-nitrophenyl phosphate to p-nitrophenol, and monitored by following absorption at 405 nm, and conversion to enzyme activity was made using the p-nitrophenol standard absorption curve.

2.2.6.2. In vivo study

2.2.6.2.1. Animal and surgical procedures

This study was conducted in accordance with the regulations and approval of the Institutional Animal Care and Use Committee of Tehran University of Medical Sciences (TUMS). 10-week-old male Sprague–Dawley rats with an initial weight of 250-290 g were purchased from Pasteur Institute of Iran for the in vivo study. Animals were anesthetized by means of intramuscular injection using ketamine (80 mg kg–1) and xylazine (10 mg kg–1). An incision was made in the anterior region of the calvarium and a 5 mm diameter critical sized full thickness bone defect was prepared using a trephine drill under continuous sterile saline irrigation. For the in vivo study the prepared calcium phosphate cement were implanted within the calvarium defect. Also, defects without implanted cements were employed as negative controls. Soft tissues were sutured to achieve primary closure. Four weeks after implantation the animals were sacrificed. The area of the original surgical defect and the surrounding tissues were removed en bloc and fixed in 10% neutral buffered formalin solution and then decalcified in 10% (v/v) nitric acid. Tissues were embedded in a paraffin block and then serial sectioned using a microtome.

2.2.6.2.2. Histological analysis and histomorphometry

For histologic assays, tissues were embedded in a paraffin block and subsequently serial-sectioned using a microtome with approximately 4–6 μm thickness and mounted on microscope slides. Slides with tissue sections were de-paraffinized and hydrated through series of xylene and alcohol. The tissue slides were stained with hematoxylin & eosin (H&E) and observed under an optical microscope for the histological observation.

2.2.6.2.3. Radiographic analysis

Radiographs were taken using an X-ray machine (Intra Planmeca-Helsinki, Finland), after the four weeks surgery after sacrificing. The percentage of bone formation within the defects was evaluated blindly and independently by two observers.

2.2.7. Statistical Analysis

Data values obtained in the experiments were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS software. Differences in the properties of the CPC were stablished by Fisher's least significant difference (LSD) mean discrimination test, using $P<0.05$ as level of significance.

III. Results and Discussion

3.1. XRD analysis

According to diffraction patterns of the prepared samples, TTCP and HA peaks were observed between $0°\leq 2\theta \leq 80°$ with no additional phase such as CaO, because TTCP obtained by means of quenching method at 1500 ºC after 12 h instead of furnace-cooled method that the powder obtained by the latter method contains TTCP, HA and free CaO from decomposition of TTCP [27]. As a result, the optimum process to obtain the high contents of TTCP and remove CaO phase is a quenching after holding 12 h. In additional, it can be concluded that all the DCPD has transformed into HA in the synthesized cement (see Fig. 1). The proportion of powder to liquid is a significant features of cement due to impact both bioresorbability and rheological properties [28]. According to the XRD patterns in Figure 1b, it can be seen that by increasing the ratio of Powder/Liquid, crystallinity of the prepared cement has also increased that represents an increase crystallinity of HA in comparison with apatite cements are formed in an aqueous environment and have a precipitated poorly crystalline HA as the end product [28]. It is commonly believed that HA contents in the starting TTCP powder impact the precipitation or nucleation for HA new phase owing to act HA as a seed material and accelerate the reaction with the hardener.

The substitution of strontium instead of calcium can alter the crystal structure, including some structural disorder that this matter can be seen in Fig. 2 as with incorporation of Sr ions, well-defined and sharp peaks was observed that in agreement with a high degree of crystallinity of cement reported by authors [29, 30]. Although, the broadening is more obvious for the samples with smaller strontium contents because there exists a greater difficulty for HA to host the larger Sr ion that for HA-containing Sr to host the smaller calcium ion with respect to their ionic radii (Ca2+ ionic radius = 0.100 nm; Sr2+ ionic radius= 0.118 nm) [29]. Additionally, it is evident that a shift of the diffraction peaks to lower 2θ values with Sr addition, representing a gradual increase in d-spacing [30]. Meanwhile, in the samples with no strontium hydroxyapatite (Sr-HA), no significant increase in crystallinity was observed.

3.2. FTIR analysis

In Figs. 3 and 4, FTIR spectra of the prepared CPC with 3 wt% Sr-HA with different P/L ratios (0.83, 1.25, and 1.55) have been shown. As it can be seen in these figures, there was no considerable difference between CPC with and without strontium.

Analyses of the spectra recorded for each cement are as follows:

As is clear from Figs. 3 and 4, FTIR spectra of the three samples are similar. The FTIR analysis determines the functional groups of the samples and since the functional groups are similar in all three prepared cements so this similarity is quite expected. In other words, the FTIR spectra of the three samples follow a single model. The Bands in the range of 3488 to 3497 cm-1 spectral of the three types of cements, are related to vibration of adsorbed water and presence of hydroxyl groups. Incorporation of Sr ion instead of Ca ion leads in decreasing of the relative intensity of the bands of OH- stretching and vibration modes as well as OH- stretching mode shifts to high wave number, on the contrary, OH- liberation band shifts to lower wave number, in agreement with results reported for Ca-HA and Sr-HA [31]. The bands 1640 to 1643 cm-1are related to absorption of H2O. The bands at 512-517 cm-1, 520-530 cm-1 and 1010-1034 cm-1 are related to V2PO4-3, V3PO4-3 and

V1PO4-3 respectively that could be due to presence of calcium phosphate compounds (TTCP, DCPD and HA) in the samples that in comparison with FTIR results obtained from Bigi et al. [29] for pure Ca-HA showed that phosphate groups shift to lower wave number with incorporation Sr ions and forming Sr-HA. The predominant factor leads to shift in the internal PO4 frequencies to lower energies is reduced anion-anion repulsion concomitant with an increased anion-anion separation on increasing cation radius. The influence of Sr in shifting phosphate group attributed to the increasing mean dimensions of the cation [29] and indicating an increase of disorder in the HA structure around phosphate sites as Sr is incorporated. Also, these bands can also be related to formation of hydroxyapatite by the reaction between the liquid phase and powder phase of the cement. Also, the observed bands at 883-881 cm-1 are related to carbonate groups that are due to the absorption of carbonate from the environment.

3.3. Thermal analysis

Thermal analysis indicates that weight reduction of the samples follows the following manner (see Fig. 5):
The first stage weight loss occurs at 25 to 280 °C is related to evaporation of water. Then, it experienced a slight weight loss in the temperature range between 280 to about 375 ° C which can be related to the crystallization of the powders from the amorphous [32]. The third stage (375 to 550 ° C) is related to Reduction of hydroxyl groups with decomposition of HPO4-2 anions to pyrophosphate anions P2O7-4. The fourth stage from 550 to 880 ° C is attributed to release of CO2 gas during endothermic decomposition of CO3-2 or can be ascribed to the decomposition of pyrophosphates to biphasic mixtures [33].
P2O74- → 2PO43- + H2O

3.4. SEM observations

The SEM micrographs of CPC and CPC with wt% Sr-HA are given in Fig. 6. This figure indicate that prepared CPC with increasing P/L ratio, the formation of hydroxyapatite crystals arising from the interaction of solid and liquid phase of cement was decreased. Of course, incorporation of Sr within Ca site culminates in a dramatic increase in crystallinity of hydroxyapatite that was in agreement with XRD results which can be attributed to structural changes. Strontium atoms can occupy both M (1) and M (2) sites of the apatite structure that depending on the amount of strontium, at very low Sr contents the occupancy of M (1) is more probable and the samples containing > 10 Sr atom% found a preferential Sr occupancy at M (2) sites [29]. M (2) sites allow a better accommodation of the bigger Sr atoms, whereas in the M(1) Site metals are hardly aligned in columns parallel to the c-axis. This suggests that the driving force in the distribution Sr is the optimization of metal-oxygen interaction, that is, due to the longer M(1)-O mean distances which allows the accommodation of a larger cation. With increase of the number of bigger ions, the repulsion between atoms in the M (1) Site would trigger an enlargement of the c-axis, furthermore, the larger amount of distribution cause a significant discontinuity in the cell parameter variation [34]. It was also reported that within each specific Sr occupancy, the relationship between the excess energies Ex of the solid solution Sr-HA-X structures and sites for substitution of Sr is Ex site (1) < Ex mixed sites < Ex site (2), indicating that Sr incorporation at site 1 is energically more favorable than at mixed sites followed by site 2 [35].

3.5. Setting time

Setting time of the prepared CPC with 3 wt% Sr-HA with different powder to liquid ratio is shown in Table 1 that indicating with an increase powder to liquid ratio, setting time reduced. The relative solubility of phases of TTCP and DCPD considered as a main driving force of calcium phosphate cements setting time so that in first two phases of TTCP and DCPD partially dissolved in liquid phase, consequently, precipitating HA according equation (1):
2Ca4(PO)2O + 2CaHPO4 → Ca10(PO4)6 (OH)2 (1)
Applying Na2HPO4 as a liquid phase to prepare cement leads to increase the rate of formation oh hydroxyapatite and decrease setting time. In other words, the higher L/P ratios, raise the working time of the mass which delays the super saturation of hydrate phases, which in apatite cement is expected to be hydroxyapatite and this leads in longer setting time of the cement [36].

3.6. Biological evaluations

3.6.1. In vitro study

3.6.1.1. MTT assay

Cytocompatibility of the prepared cements were assessed using MTT assay. The results of MTT assay are shown in Fig. 7. This result suggests that after 14 days of incubation, the cell viability decreased for all samples, but this reduction is insignificant for all of them. Also, the results showed that an increase of 3 wt. % of nanostrontium hydroxyapatite (Sr-HA) has increased the biocompatibility of the prepared cements. Finally it can be concluded that the prepared cements have proper biocompatibility and are suitable for use as bone cement.
It has been proved that Sr ions can stimulate cellular responses ans subsequently go up rate of cell viability process as well as the influence of strontium ions on viability of osteoblastic cells was showed to be dose-dependent, owing to the lower viability rate of the osteoblasts on calcium phosphate cements compared with other Sr-hydroxyapatite cements that it was in agreement with other studies. Hence, Sr in hydroxyapatite cement can be encouraged new bone growth [30, 37]. Although, incorporation of Sr enhances the cell proliferation, but Sr concentration is still controversial. Park et al [38] found that Sr ions release at 103-135 ppb increased the differentiation of osteoblast. Also, others results suggest that Sr alone at concentration between 1.21 and 3.24 does not impact the proliferation of osteoblast cells [30].

3.6.1.2. Alkaline phosphatase

Measurement of intracellular alkaline phosphatase activity is considered as one of the criteria in osteoblast activity. Fig. 8 shows the results of the ALP activity of the different prepared cements after 3 and 7 days of incubation. It can be concluded that with the addition of 3 wt. % of Sr-HA alkaline phosphatase activity significantly increased, which may be due to the presence of strontium ions, because strontium ions increase the activity of bone cells. The cements with P/L ratio of 1.25 had the highest level of alkaline phosphatase activity compared to P/L ratios of 1.55 and 0.83. Therefore, the P/L ratio of 1.25 was selected as optimum ratio in CPC.
It is noteworthy that the biology activity of Sr-containing

cement is also attributed to the influences on crystallinity and expansion of its crystal lattice due to the larger size of Sr compared to Ca. In addition, according Figure (SEM), the samples containing Sr consisted of larger, needle-like crystals, as a result, the small tightly entangled crystal can provide more nucleation sites for the formation of apatite crystals [39, 40].

3.6.2. In vivo study

3.6.2.1. Histological analysis and histomorphometry

In this study, the in vivo bone tissue response towards three samples was evaluated by means of histological examination. The study was aimed to determine the effect of Sr on the biological behavior cements. Figure 8 shows the optical micrograph of in vivo examination of 3 wt% Sr-HA/CPC, CPC and control after 4 weeks. According to the obtained results, no inflammation, tissue necroses or tissue rejection was observed after implantation. Generally, during the in vivo process at 4 weeks post-operation, fibrous connective tissue and blood vessels grow into the macropores, contributing to the early fixation of the samples.

CPC gradually dissolve in the body (in vivo), seeding new bone formation as they release calcium and phosphate ions into the biological medium [41]. The formation of the dynamic interface between CPC and host bone is believed to result from a sequence of events involving interaction with cells and the formation of a carbonate HA by a dissolution/precipitation process [41]. CPCs support bone formation while they partially dissolve and degrade in the body. It is worth mentioning that these properties are related to their physical, chemical and microstructural characteristics [41].

Also, as it can be observed in Fig. 9, histomorphometric analysis displayed a statically dramatic increase in the bone formation for the cement containing strontium. Sr-cement treated animals showed a statistically higher the formation of new bone in comparison with the defect areas filled with cement without Sr and empty defect in the defect region. Thus, there was more bone formation at the bone biomaterials interface region for the Sr-cement compared to cement which was a significant. It should also be noted that the formation of bone at both the periphery and center of fracture defect area for all the samples have been seen which can be attributed to the local release of Sr from cement can able to positively effect osteogenesis, immunohistological and molecular-biological evaluation could confirm the enhanced the formation of new bone activities in Sr-containing cement with enhanced expression of BMP2, osteocalcin and OPG in comparison with calcium phosphate cement and the empty defect control. Also, the authors reported that strontium can be released into the local milieu of osseo-integrating implants to accelerate bone ingrowth into the implant surface. Furthermore, it is possible that strontium´s biological activity to encourage new bone formations preserved within the cement [42, 43]. The role of Sr in enhancing new bone formation is that the strontium uptake in bone may increase the bone volume by raising the extent of bone formation sites and decreasing bone resorption, with no affecting the rate of the formation of bone and changing the bone mineralization [39].

3.6.2.2. Radiographic analysis

Strontium-containing cement is a radiopaque material that can be observed under X-ray, thus, Sr-HA acted as a radiopaque material, and no other materials needed to be added to the bone cement to allow radiographic imaging. No inflammatory response and no necrosis were found in the rats injected with Sr-HA cement (see Fig. 10). Meanwhile, the similarity of minerals in Sr-HA cement and bone promote fusion of the materials and bone completely during new bone formation, also, development of blood vessels suggests that the bone metabolism was active. As a consequence, Sr-HA bioactive cement has the potential to be utilized in vertebroplasty to treat osteoporotic fractures [44].

IV. Conclusions

In conclusions, XRD analysis demonstrated that increasing the ratio of Powder/Liquid (P/L), crystallinity of the prepared cement has increased. The substitution of strontium instead of calcium in CPC can also alter the crystal structure, including some structural disorder. However, in the CPC with no strontium hydroxyapatite (Sr-HA), no significant increase in crystallinity was observed. SEM observations discovered CPC with increasing P/L ratio, the formation of hydroxyapatite crystals arising from the interaction of solid and liquid phase of cement was reduced. Also, incorporation of Sr within Ca site culminates in a considerable increase in crystallinity of hydroxyapatite. However, in this work, the CPC with 3 wt% Sr-HA bone regeneration group has been shown to function to enhance the MTT assay and ALP activity in vitro and bone regeneration in vivo. Eventually, from our findings, we suggest that CPC with proper strontium additives can efficiently increase the rate of bone regeneration compared with CPC without strontium.

V. Declaration of Conflicts of Interest

The authors report no declarations of interest. The authors are responsible for the content and writing of the paper. Authors thank Dental Biomaterials Department, School of Dentistry, Tehran University of Medical Sciences (TUMS), Tehran, Iran, for financial support. This paper is based on a PhD thesis submitted in Dental Biomaterials Department, School of Dentistry, Tehran University of Medical Sciences (TUMS).

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FIGURE CAPTIONS

Figure 1. XRD pattern of prepared CPC with different P/L ratio

Figure 2. XRD pattern of prepared CPC with 3 wt% Sr-HA with different P/L ratio

Figure 3. FTIR spectra of prepared CPC with different P/L ratio

Figure 4. FTIR spectra of prepared CPC with 3 wt% Sr-HA with different P/L ratio

Figure 5. TG-DTA patterns of CPC with 3 wt% Sr-HA (P/L=1.25) heated from 30 oC to 800 oC with heating rate 10 oC/min

Figure 6. SEM micrographs of prepared CPC (a) with and (b) without 3 wt% Sr-HA with different P/L ratio (from left to right: P/L=0.83, 1.25 and 1.55)

Figure 7. Cell proliferation of SAOS-2 cells proliferated on the CPC with and without 3 wt% Sr-HA with different P/L ratio along with negative control after incubation for 7, and 14 days

Figure 8. ALP activity test for SAOS-2 cells proliferated on the CPC with and without 3 wt% Sr-HA with different P/L ratio along with negative control after incubation for 7, and 14 days

Figure 9. Optical micrographs of (a) control, (b) 3 wt% Sr-HA/CPC (P/L=1.25) and (c) CPC and control in implant

site for 4 weeks implantation

Figure 10. Radiography image of 3 wt% Sr-HA/CPC (P/L=1.25), CPC and control in implant site for 4 weeks implantation (L: CPC, R: 3 wt% Sr-HA/CPC (P/L=1.25) and F: Control)

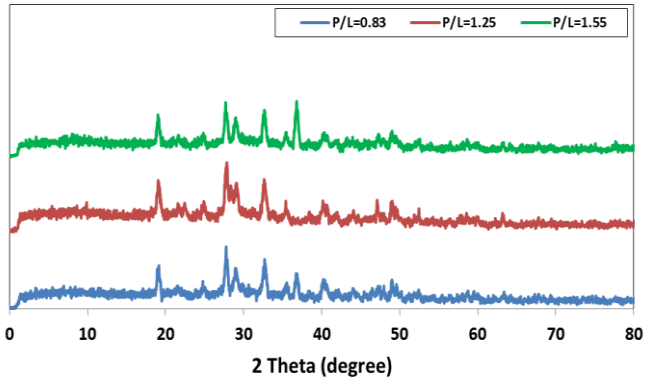
TABLE

Table 1. Setting time of the prepared CPC with 3 wt% Sr-HA with different P/L ratio

sample	P/L ratio	Setting time (min)
1	0.83	28.7±0.4
2	1.25	9.3±0.1
3	1.55	2.2±0.2

FIGURES

Figure 1



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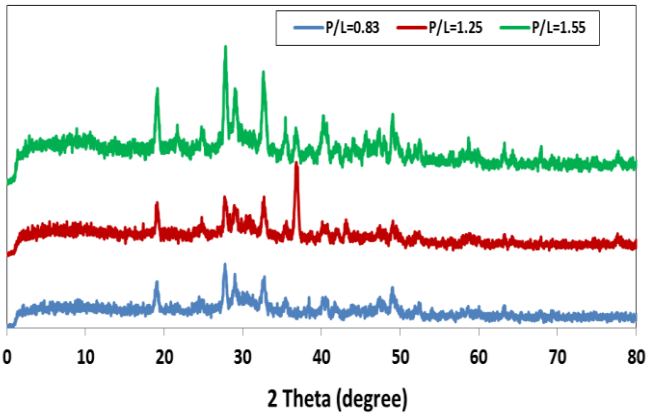

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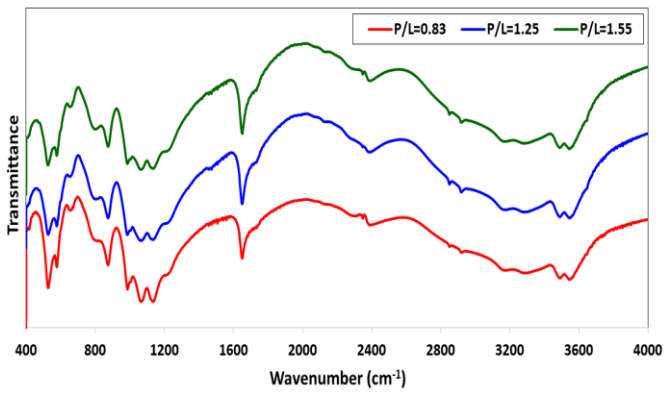

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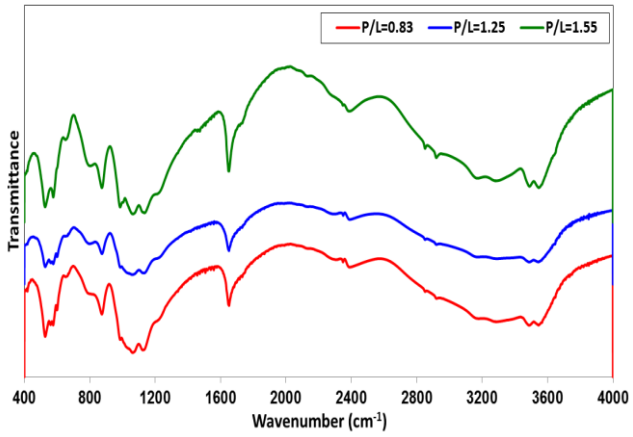

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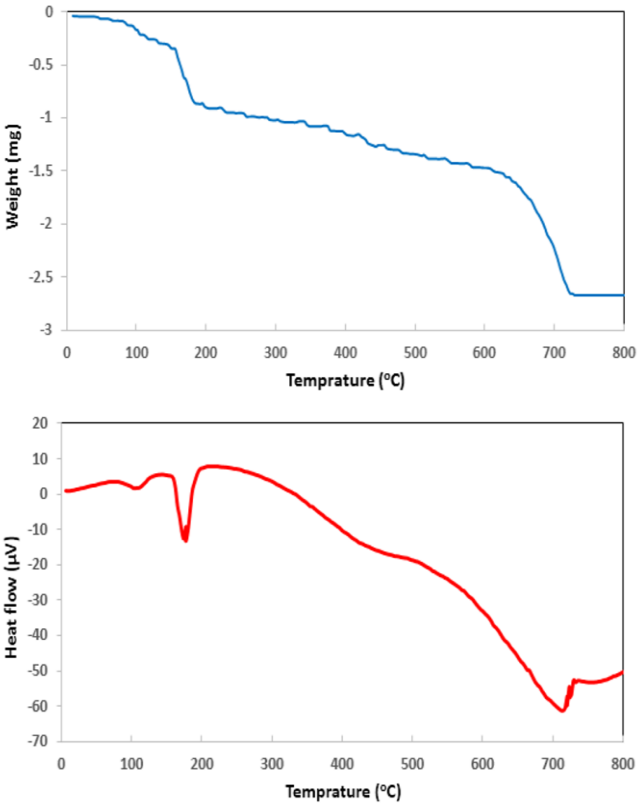

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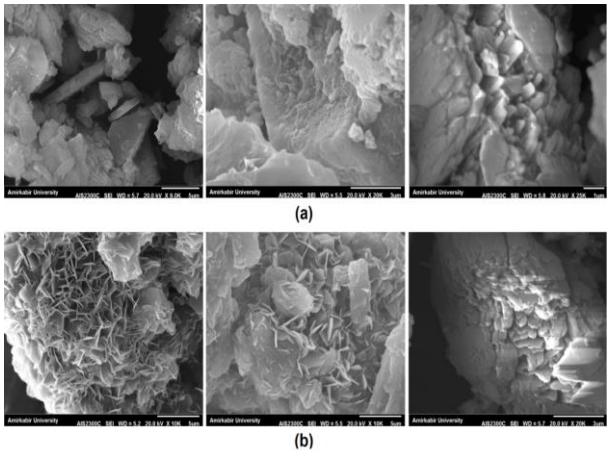

Figure 7

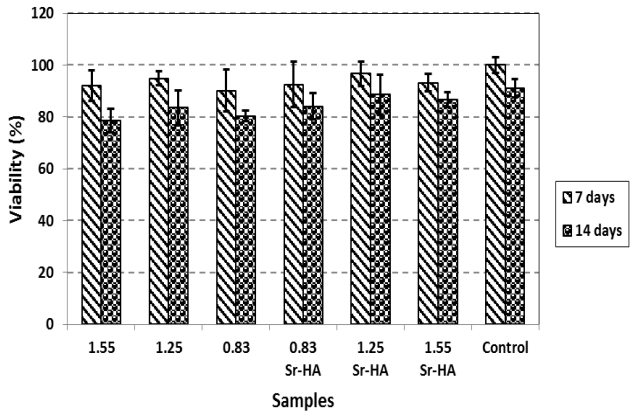

Figure 8

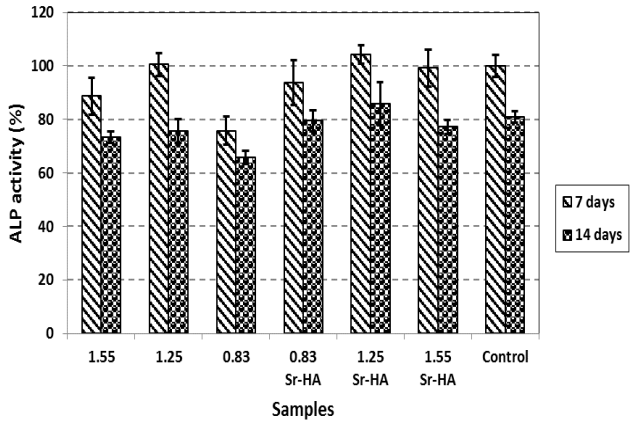

Figure 9

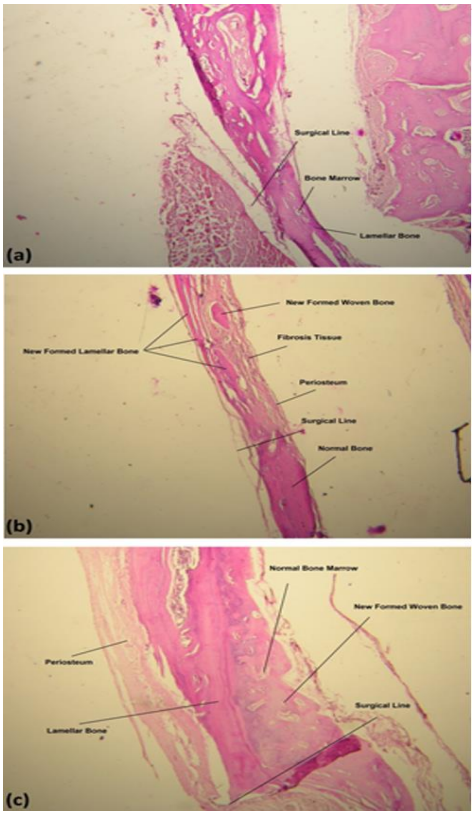

Figure 10